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Cutaneous Responses to 12-Hydroxy-5,8,10,14-eicosatetraenoic Acid (12-HETE)

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The responses to 12-HETE in normal human skin have been investigated by means of intradermal and topical administration in 15 subjects. Intradermal infusion of 12-HETE produced a neutrophil polymorphonuclear and mononuclear infiltrate in the dermis. Topical administration resulted in a dose-related erythematous response to 200 ng–50 μg. This was accompanied by a neutrophil and mononuclear dermal infiltrate at 6 and 24 h after application. In addition, collections of neutrophils were present in the epidermis in 4 of 10 subjects biopsied at 6 h and in all patients biopsied 24 h after topical application. Intradermal and topical application of 9-hydroxyoctadecadienoic acid (9-HODD), a chemically similar but chemokinetically inactive substance, did not produce neutrophil infiltration of the epidermis, nor did the chemical irritant nonanoic acid. The results suggest that the cellular infiltrates produced in vivo in humans by 12-HETE are due to its chemoattractant properties and are not the result of a nonspecific inflammatory response.

The arachidonate lipoxygenase product 12-S-hydroxy 5,8,10,14-eicosatetraenoic acid (12-HETE) is formed via the action of 12-lipoxygenase on arachidonic acid.

In vitro 12-HETE was demonstrated to be chemotactic for polymorphonuclear leukocytes [1]. Subsequently it was shown that partially purified 12-HETE was chemotactic for eosinophils, with substantial activity for neutrophils and less activity for mononuclear cells [2]. Further in vitro studies demonstrated that 12-HETE was both chemokinetic and chemotactic for human eosinophil and neutrophil polymorphonuclear leukocytes [3]. These observations were confirmed by Ford-Hutchinson et al [4] and Palmer et al [5] who also demonstrated that in vitro 12-HETE was approximately 100 times less potent as a chemoattractant than leukotriene B_4. 12-HETE has also been shown to stimulate human polymorphonuclear leukocyte aggregation [6].

The results of previous studies of the in vivo properties of 12-HETE have yielded conflicting results. 12-HETE injected i.p. into guinea pigs at doses of 4 and 8 μg in 0.4 ml of Hanks' balanced salt solution induced an influx of eosinophils and neutrophils with peak levels at 30 min and 5 h, respectively. At 5 h the neutrophils were the predominant cells [7]. The intradermal injection of 1 μg 12-HETE in 100 μl Tris buffer, pH 7.5, into rabbit skin enhanced the infiltration of leukocytes, predominantly polymorphonuclear leukocytes, at 4 h [8].

However, intradermal injection of 12-HETE into rabbit skin at doses up to 1 μg/100 μl Tris buffer did not significantly increase the leukocyte infiltration compared with that produced by control injection of Tris buffer at times 1–4 h after injection into rabbit skin [9]. Furthermore, the introduction of 5 μg 12-HETE in 0.9% sodium chloride into the anterior chamber of the rabbit eye evoked no leukocyte infiltration in the aqueous humor after 4 h [10]. To our knowledge there have been no further investigations of the in vivo effects of 12-HETE.

Elevated levels of lipoxygenase products including 12-HETE have been found in psoriasis. Hammarstrom et al [11] found that the ratio of 12-HETE in the involved compared to the uninvolved skin of patients with psoriasis was approximately 100. We have previously shown that 12-HETE is present in biologically active amounts in extracts of lesional psoriatic skin [12–15] and semiquantitative analysis by gas chromatography-mass spectrometry showed a mean of approximately 1 μg 12-HETE per 100 mg psoriatic scale [15]. Moreover, elevated levels of 12-HETE have been found in UV-irradiated normal human skin and anthralin-treated clinically normal skin of patients with chronic plaque psoriasis [16].

In view of the chemokinetic and chemotactic properties of 12-HETE and its presence in psoriasis and inflamed skin, we considered that an investigation of its in vivo properties in human skin was indicated. We have therefore investigated the in vivo effects of intradermal and topical administration of 12-HETE in normal human skin.

MATERIALS AND METHODS

Chemically and biologically synthesized 12-HETE were used in the intradermal experiments. In the investigation of the effects of topical administration of 12-HETE, chemically synthesized 12-HETE was used.

Biologically synthesized 12-HETE was prepared by the method of Hammarstrom et al [11]. Arachidonic acid (150 μM) was incubated with washed human platelets and 12(S)-HETE was then isolated by silicic acid chromatography and straight-phase high-performance liquid chromatography (HPLC). 12(S)-HETE prepared in this manner was administered to the human subject from whom the washed platelet preparation was obtained. Chemically synthesized 12(R,S)-HETE was prepared by photooxidation of arachidonic acid [15]. Arachidonic acid (100 mg) in solution in methanol containing 0.01% methylene blue was irradiated with 3×500-W incandescent bulbs (PF 318 P2/3) for 2½ h. Oxygen was bubbled through the solution which was cooled below 10°C. The hydroperoxides so formed were reduced with sodium borohydride and the products subjected to preliminary purification by reversed-phase gel partition chromatography using Lipidex 5000. The monohydroxy fatty acid fraction was subjected to straight-phase HPLC by which the positional isomers, including 12(R,S)-HETE, were isolated. 9(R,S)-Hydroxyoctadecadienoic acid (9-HODD) was prepared by photolysis of linoleic acid (100 mg) in a manner identical to that by which 12(R,S)-HETE was prepared from arachidonic acid [15]. Nonanoic acid (pelargonic acid, $CH_3(CH_2)_7COOH$ was obtained from the Sigma Chemical Company, Poole, Dorset, U.K. Phosphate-buffered saline, 0.04 M sodium phosphate, 0.154 M NaCl, pH 7.3 (PBS) was prepared under sterile conditions.

Intradermal injections were administered by a 25-gauge needle.

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Abbreviations:
12-HETE: 12-hydroxy-5,8,10,14-eicosatetraenoic acid
HPF: high-power fields
HPLC: high-performance liquid chromatography
9-HODD: 9(R,S)-hydroxyoctadecadienoic acid
PBS: phosphate-buffered saline, 0.04 M sodium phosphate, 0.154 M NaCl, pH 7.3

Intradermal infusions were administered by means of a portable battery-operated MS26 syringe driver (Graseby Dynamics Ltd., Watford, Herts., England). This allowed the concurrent administration of solutions from 2 separate insulin (1.0 ml) syringes attached to 27-gauge butterfly cannulae. Biopsies were obtained by means of sterile disposable punch biopsies (3 mm). Local anesthesia was achieved by 2% lignocaine without adrenaline. Biopsy specimens were fixed in formol saline, and processed for light microscopy. Sections (4 μm) were stained by hematoxylin and eosin and viewed on a Wild microscope (10× eye piece, 20×, 40× objectives).

The overall histologic pattern of the infiltrate produced in response to 12-HETE was determined at low magnification. In biopsies from sites of intradermal administration of 12-HETE a morphometric analysis of the dermal infiltrate was performed as follows: the densest part of the dermal infiltrate was determined and 2 independent observers then counted the numbers of neutrophils, mononuclear cells, and vessels in each of 10 high-power fields (HPF) (400× magnification) of this zone in each of 2 nonadjacent sections from each biopsy. Sections from test vs control sites were examined blind by the observers. The results were expressed as the number of cells or vessels per HPF. The significance of the differences in numbers of cells in sections from test and control sites was determined by paired *t*-tests.

Analysis of regression was employed to determine whether the increase in number of inflammatory cells was quantitatively related to the vascularity of the field. Analyses of variance were performed to establish whether the nature of the control substance quantitatively affected the infiltrate.

In biopsies obtained from sites after topical administration of 12-HETE, a morphometric analysis of the epidermal infiltrate was performed. Morphometric analysis of the neutrophil epidermal infiltrate was achieved by 2 independent observers. Cells were counted in adjacent HPF along the full length of the epidermis of 2 nonadjacent sections from each biopsy. In order to allow for variation in the length of the histologic sections, the results were expressed as the number of neutrophils per 10 HPF.

Subjects

All subjects were healthy adults with normal skin.

Intradermal Administration

Five and forty micrograms of chemically synthesized 12-HETE were injected intradermally into the forearm skin of 3 subjects. The site was observed for 8 h and then biopsied.

Biologically synthesized 12-HETE was administered by intradermal infusion with PBS as control to 2 patients. Chemically synthesized 12-HETE was administered to 2 patients with PBS as a control and to a further 2 patients with 9-HODD as control. 9-HODD was chosen as a control because of its chemical similarity to 12-HETE and its lack of leukocyte chemokinetic activity in the agarose microdroplet assay which we employ to determine biologic activity [15]. Solutions of 12-HETE and 9-HODD (both 0.39 mM) were made up in PBS. These and PBS alone were infused at a rate of 40 μl/h for 8 h, at the end of which the inflammatory response was observed. The total dose of 12-HETE was 40 μg. Biopsies of test and control sites were then performed. The inflammatory infiltrates were characterized histologically and the intradermal infiltrate subjected to morphometric analysis as above.

Topical Administration

Chemically sythesized 12-HETE was administered topically in doses of 200 ng–50 μg in 5 μl ethanol to a 0.25-cm^2 area of the skin of the ventral surface of the forearm. The ethanol was evaporated and the residue occluded for 6 h (n = 15). The erythematous response was observed for 48 h and repeated measurements at different time intervals of the 2 largest diameters of erythema to the nearest 1 mm were made by a transparent rule at each application site in order to establish a dose response. 12-HETE (20 μg) in 5 μl ethanol was then applied to the ventral surface of the skin of the forearm of 15 subjects. Biopsies were taken at either 6–8 h (10 subjects) or 24 h (5 subjects). An equimolar solution of 9-HODD in 5 μl ethanol and undiluted nonanoic acid were similarly applied to the forearm skin as controls (3 and 2 subjects, respectively). In addition, a 40% solution of nonanoic acid in propan-2-ol was applied to the ventral surface of the forearm under occlusion for 24 h and then biopsied. Biopsies of control sites were performed 24 h after topical applications. Nonanoic acid was chosen as an additional control because of its chemical irritant properties [16].

RESULTS

Intradermal Administration by Injection

The intradermal injection of 12-HETE (5 and 40 μg) produced no visible inflammatory response after 8 h observation and histology of the injection sites at 8 h revealed only a sparse perivascular infiltrate in the dermis.

Intradermal Administration by Infusion

Erythema of approximately equivalent extent was evident at both test and control infusion sites at the completion of the infusion. Histology of the sites of infusion of both biologically and chemically synthesized 12-HETE revealed an infiltrate around vessels and randomly distributed in the dermis, whereas histology of PBS and 9-HODD infusion sites revealed only a slight increase in perivascular inflammatory cells. The infiltrate in the 12-HETE infusion sites consisted of neutrophil polymorphonuclear leukocytes and mononuclear cells (Fig 1). Morphometric analysis revealed a mean increase of 12.2 neutrophils and 6.5 mononuclear cells per HPF (for original data see Table I) in 12-HETE infusion sites compared with control infusion sites. Paired *t*-tests revealed these differences to be significant ($p = 0.03$) in each case. Analysis of regression revealed no evidence of a correlation between the numbers of cells of each type and the number of vessels per HPF (Table I), thus indicating that the density of the cellular infiltrate was not merely a reflection of the vascularity of the field.

There was no evidence of a quantitative or qualitative difference between the numbers of cells present in the infiltrates produced by biologically or chemically synthesized 12-HETE when these were compared in relation to each other with PBS as control infusion by analysis of variance. Analysis of variance showed that the increase in population density of infiltrating cells did not significantly differ irrespective of whether PBS or 9-HODD was used as a control.

Topical Administration

A dose-related erythematous response was obtained when 200 ng–50 μg 12-HETE was applied to forearm skin (Table II).

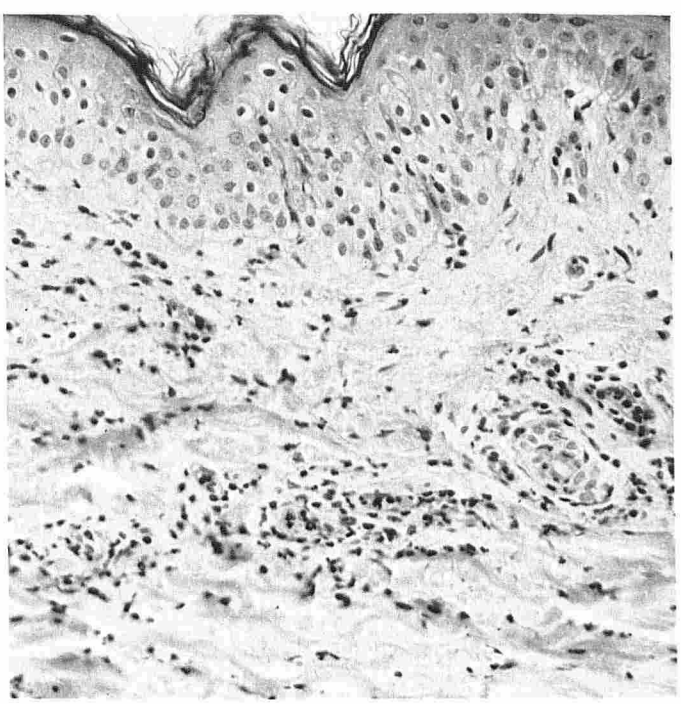

FIG 1. Photomicrograph of 12-HETE infusion site showing neutrophil polymorphonuclear leukocytes and mononuclear cell infiltrates around vessels and randomly distributed in the dermis.

TABLE I. *Mononuclear and neutrophil cell and blood vessel counts in skin infused with 12-HETE and control substance*

Subject number	Active substance	Number of neutrophils per HPF	Number of mononuclear cells per HPF	Number of vessels per HPF	Control substance	Number of neutrophils per HPF	Number of mononuclear cells per HPF	Number of vessels per HPF
1	Biologically synthesized 12-HETE	17.3	10.1	1.7	PBS	0.8	0.7	1.0
2	Biologically synthesized 12-HETE	16.5	4.9	0.9	PBS	1.2	0.9	0.9
3	Chemically synthesized 12-HETE	16.8	9.2	1.0	PBS	0.5	0.2	0.5
4	Chemically synthesized 12-HETE	15.8	4.9	0.6	PBS	0.7	0.6	1.2
5	Chemically synthesized 12-HETE	6.8	9.7	0.5	9-HODD	0.4	0.6	1.1
6	Chemically synthesized 12-HETE	6.3	5.4	1.2	9-HODD	0.8	2.4	0.9

Mean numbers of cells and vessels per HPF (n = 10) present in the infiltrates at the test and control infusion sites at the completion of 8-h infusions.

TABLE II. *Area of erythema (mm^2) after topical application of 12-HETE*

Subject	Area of erythema at 6 h μg dose of 12-HETE					Area of erythema at 24 h μg dose of 12-HETE				
	2	5	10	20	50	2	5	10	20	50
1	33.18	33.48	44.18	63.62	70.88	9.62	19.63	38.48	44.18	70.88
2	12.57	19.63	23.76	33.18	78.54	7.07	9.62	9.62	15.90	19.63
3	19.63	12.57	28.27	19.63	23.76	0.00	0.00	0.00	0.00	0.00
4	19.63	63.62	56.74	78.54	95.03	56.74	44.18	28.27	23.76	VFDE
5	FE	VFE	VFE	19.63	28.27	0.00	0.00	0.00	0.00	28.27
6	19.63	23.76	38.48	70.88	70.88	7.07	7.07	15.90	19.63	38.48
Mean	14.16	22.38	25.49	35.77	49.41	12.23	12.23	12.73	14.78	19.79
± SEM	12.84	24.76	22.96	29.70	37.25	22.19	16.73	16.73	16.46	27.27

Key: FE = faint erythema; VFE = very faint erythema; VFDE = very faint diffuse erythema.

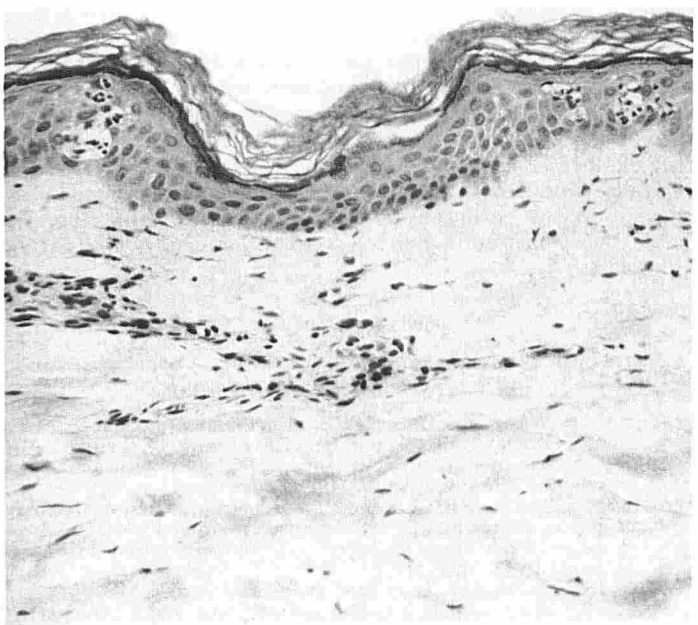

FIG 2. Photomicrograph of histologic appearances 6 h after topical application of 12-HETE, showing a neutrophil and mononuclear cell infiltrate at the dermal-epidermal junction and discrete collections of neutrophils in the epidermis.

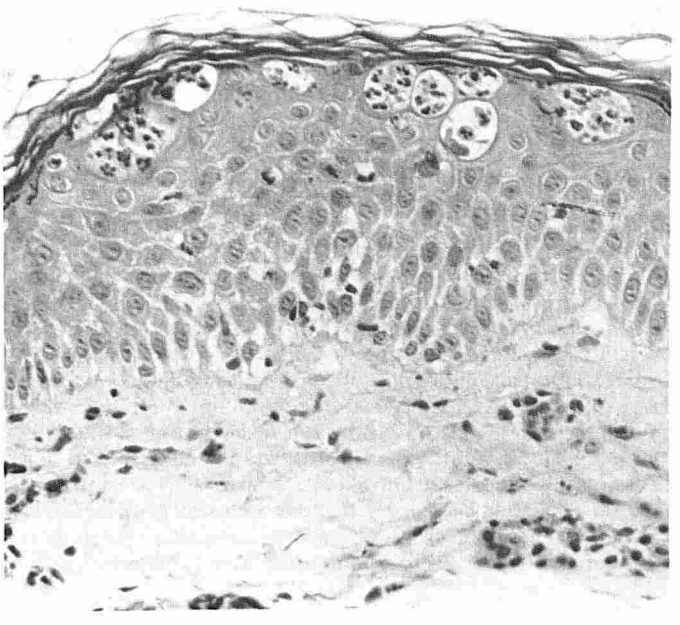

FIG 3. Photomicrograph of histologic appearances in a biopsy taken 24 h after topical application of 12-HETE, showing discrete collections of neutrophils in the epidermis.

No pustules were seen but in 3 subjects the erythema was accompanied by edema. At sites of application of doses of 20 and 50 μg, erythema lasted for 15–24 h in the majority of subjects. There was, however, considerable intersubject variation in the magnitude of the erythematous response at 24 h (Table II). No visible reaction was seen after 9-HODD application at 6 and 24 h. Following the application of undiluted nonanoic acid, palpable erythema without vesiculation or pustulation was seen at 6 and 24 h. Nonpalpable erythema was present after 40% nonanoic acid solution was applied to the skin under occlusion for 24 h.

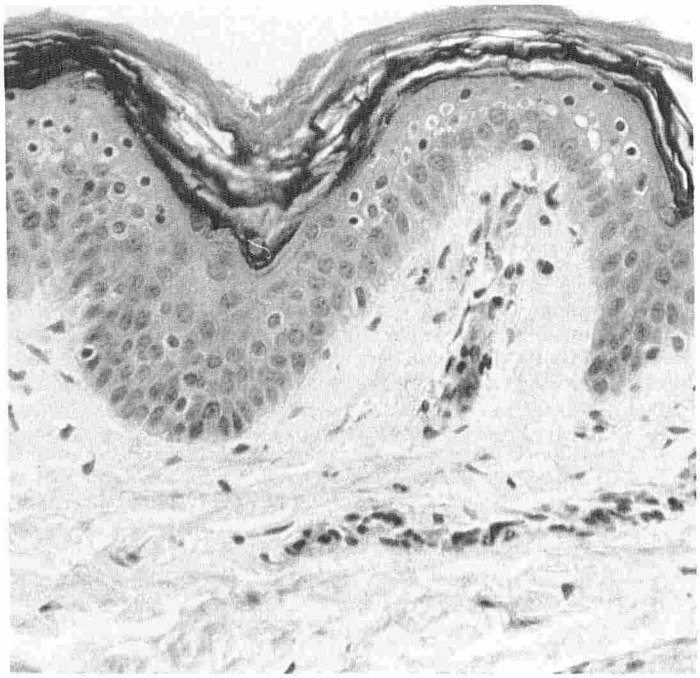

FIG 4. Histologic appearances after topical application of undiluted nonanoic acid showing necrolytic changes in the malpighian layer of the epidermis.

In all 10 biopsies taken 6–8 h after the topical application of 20 μg 12-HETE, dermal neutrophil and mononuclear cell infiltrates were seen. These were of maximal intensity around the upper dermal vessels, and in places the infiltrate impinged on the dermal-epidermal junction. In 4 of the 10 biopsies discrete collections of neutrophils could also be seen in the epidermis (Fig 2).

In all 5 biopsies taken 24 h after the application of 12-HETE there was a neutrophil and mononuclear cell infiltrate in the dermis. This was accompanied by an intraepidermal neutrophil infiltrate, often occurring in the form of discrete accumulations of neutrophils in a subcorneal position (Fig 3).

There was considerable intersubject variation in the population density of the neutrophils in the epidermis (73.9 ± 21.8).

There was no increase in the number of neutrophil or mononuclear cells in the epidermis in histologic sections from biopsies performed 24 h after topical application of 9-HODD but a sparse mononuclear cell dermal infiltrate was observed. The most striking feature of the histologic appearances resulting from the topical application of undiluted nonanoic acid was a marked necrolytic change affecting the malpighian layer of the epidermis. There was only minimal accompanying spongiosis and a sparse mononuclear cell infiltrate in the dermis (Fig 4). Histology of biopsies from sites to which a 40% solution of nonanoic acid had been applied under occlusion for 24 h showed marked epidermal necrosis and spongiosis and a sparse mixed predominantly perivascular mononuclear infiltrate in the dermis.

DISCUSSION

These results indicate that biologically and chemically synthesized 12-HETE are capable of eliciting a neutrophil polymorphonuclear and mononuclear leukocyte infiltrate when administered by intradermal infusion into normal human skin. The insignificant responses to infusions of an equimolar concentration of 9-HODD and to PBS alone indicated that these effects were not merely the result of the presence of nonspecific chemical irritants in the skin or the result of the infusion needle acting as a physical irritant to the skin.

Interestingly, in contrast to previous reports of the in vitro and in vivo effects of 12-HETE [1,2,3,5] indicating that 12-HETE was a chemoattractant for eosinophils, no evidence of eosinophil accumulation was seen in any of the biopsies obtained after administration of 12-HETE to human skin. Possible explanations for this finding are species differences in response to 12-HETE or an earlier accumulation and subsequent disappearance of eosinophils from the infiltrates by 6 h.

The failure of the linoleic acid derivative 9-HODD to produce an inflammatory response suggested that the intradermal and topical administration of 12-HETE could not be explained on the basis of a chemical irritant effect produced by the presence of a fatty acid derivative in the skin. Moreover, the neutrophil infiltrates resulting from the administration of topically applied 12-HETE were not evident in response to the topical application of the primary irritant nonanoic acid [17]. This suggests that the observed inflammatory changes result from the chemoattractant properties of 12-HETE and not from any role it might have as a chemical irritant.

Further evidence that the clinical and histologic responses observed after topical application of 12-HETE were not those of a chemical irritant comes from the study of Björnberg [18], who characterized the clinical and histologic changes 24 h after application of primary irritants to the skin. Only in severe irritant reactions characterized clinically by erythema, infiltration, and pustulation were discrete collections of polymorphonuclear leukocytes seen in the epidermis and then these were always accompanied by lymphocytes and spongiosis. Spongiosis was evident also in clinically less severe irritant reactions which were not characterized by leukocytic infiltration of the epidermis. Spongiosis was never observed in biopsies from sites following administration of 12-HETE.

The conflicting results of previous in vivo studies on the effects of 12-HETE can, perhaps, on careful review of the literature, be explained by the use of different amounts of 12-HETE and by the differences in time between in vivo administration and subsequent sampling of guinea pig peritoneal fluid [7] and rabbit skin [8,9] and aqueous humor [10]. The results of the experiments reported here indicate that 12-HETE is capable of eliciting a neutrophil and mononuclear cell infiltrate in human skin and that the infiltrates result from specific chemoattractant properties of 12-HETE. In view of the presence of 12-HETE in elevated amounts in psoriasis and experimentally produced inflammatory reactions, it may well be relevant to the pathogenesis of the neutrophil infiltrate in psoriasis and have an in vivo role as an inflammatory mediator in humans.

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